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Productivity and quality of *Brachiaria brizantha* B4 seeds in function of nitrogen doses

Produtividade e qualidade de sementes de *Brachiaria brizantha* B4 em função de doses de nitrogênio

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ABSTRACT

This study aimed to evaluate aspects of reproductive phenology, yield components and seed quality of the B4 genotype of ***Brachiaria brizantha*** *in function of nitrogen doses applied at pre-flowering. The experiment was carried out at Embrapa Beef Cattle, in a randomized block design, with seeding density of 2.59kg SPV ha^{-1} in the first year, in free growth plants. Urea was applied at pre-flowering at doses of 0, 25, 50, 75, 100, 125 and 150kg ha^{-1}. The reproductive cycle of the plant lasts 225 days, with late flowering. N doses influenced the following characteristics: number of fully expanded inflorescences, percentage of dry matter, dry biomass, number of spikelets per raceme, seed yield, weight of a thousand seeds, germination, germination speed index, viability and germination at 10 months after harvest. The maximum yield of pure seed was 144.8kg ha^{-1} for 50kg ha^{-1} N, with maximum biological potential, based on components of seed yield of 456.27kg ha^{-1}. Seeds presented high dormancy and reduced physiological quality in higher N doses.*

Key words: *fertilizer, tropical forage, seed quality.*

RESUMO

Objetivou-se avaliar aspectos da fenologia reprodutiva, componentes do rendimento e qualidade de sementes do genótipo B4 de ***Brachiaria brizantha*** *em função de doses de nitrogênio aplicado no pré-florescimento. O experimento foi conduzido na Embrapa Gado de Corte, em blocos casualizados, densidade de semeadura de 2,59kg SPV ha^{-1}, em plantas de primeiro ano e em crescimento livre. Ureia foi aplicada no pré-florescimento nas doses 0, 25, 50, 75, 100, 125 e 150kg ha^{-1}. O ciclo reprodutivo da planta foi de 225 dias, com florescimento tardio. Doses de N influenciaram o número de inflorescências totalmente emergidas, porcentagem de matéria seca, biomassa seca, número de espiguetas por racemo, produtividade de sementes puras, peso de mil sementes, germinação, índice de velocidade de germinação, viabilidade e germinação aos 10 meses da colheita. A produtividade máxima de sementes puras foi 144,8kg ha^{-1} para 50kg ha^{-1} N, com potencial biológico máximo, baseado nos componentes da produção de sementes de 456,27kg ha^{-1}. As sementes apresentaram dormência elevada e redução na qualidade fisiológica nas maiores doses de N.*

Palavras-chave: *adubação, forrageira tropical, qualidade de sementes.*

INTRODUCTION

The seeds market of tropical forage species demands high quantity and superior quality of seeds. There are few studies regarding commercial seed production of cultivars which have been available in the market for a considered period of time, as well as those which have been recently released. (DEMINICIS et al., 2010; VERZIGNASSI, 2010; LIMA, 2012).

B4 genotype is a ***Brachiaria brizantha*** derived from material originated in Ethiopia, which is located at lat. 8º12'N, long. 35º19'E, at 1610m asl, with 4.9 soil pH, and 1900mm rainfall/year (VALLE, 2015, unpublished data). Such genetic material is at pre-release stage by the breeding program of Embrapa Beef Cattle, to be released in 2017 or 2018.

Nitrogen has an important role in the production of seeds, and it is crucial in the metabolism of plants; it participates as a constituent of protein molecules, co-enzymes, nucleic acids,

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chlorophyll and other enzymes, and controls the development of plants (ABRANTES et al., 2010). The adequate supply of nutrients for plants, especially nitrogen, significantly increases seed yield and quality (CONDE & GARCIA, 1988). This nutrient has an essential role in the growth of forage grasses, and acts in tillering, in development of new leaves, and in the increase of plant vigor.

Considering that not always the characteristics of soil, climate and agronomic management necessary for the maximum seed yield coincide with those required in forage production (HOPKINSON et al., 1996; HACKER, 1999), studies on seed yield and on physiological responses of tropical forage grasses to environmental effects are extremely important (VERZIGNASSI, 2010) and currently scarce (SOUZA, 1995; FRANÇA, 2011).

The aim of this study was to evaluate some aspects of reproductive phenology, yield components and seed quality of B4 genotype of ***Brachiaria brizantha*** in function of nitrogen doses applied at pre-flowering.

MATERIALS AND METHODS

The experiment was carried out in the 2013/2014 season, at Embrapa Beef Cattle, in Campo Grande, MS, located at lat. 20º27'S, long. 54º37'W, at 530m asl. The climate is rainy tropical savanna, Aw subtype, characterized by rainy summer and dry winter. The soil type is Dystrophic Oxisol, with medium texture (32% clay).

Soil collected at a depth of 0-20cm before setting the trials had the following characteristics: 4.81 pH - H_2O; 33.45g dm^{-3} MO; 1.61mg dm^{-3} P; 0.09cmol dm^{-3} K; 0.53cmol dm^{-3} Ca+Mg; 7.03cmol dm^{-3} Al+H; 0.64cmol dm^{-3} sum of basis; 7.67cmol dm^{-3} cation exchange capacity; and 8.34% base saturation. Micronutrients presented: 85.63mg dm^{-3} Fe; 41.76mg dm^{-3} Mn; 8.73mg dm^{-3} Zn; and 3.39mg dm^{-3} Cu; 0.24mg dm^{-3} B.

Correction and soil fertilization were carried out based on the results of soil analysis, 3.37t ha^{-1} dolomitic limestone (80% PRNT) and fertilizer (on October 28th, 2013), using 500kg ha^{-1} gypsum, 132kg ha^{-1} P_2O_5(MAP), 70kg ha^{-1} K_2O (potassium chloride), 1kg ha^{-1} B (boric acid), and 0,2kg ha^{-1} Mo (sodium molybdate).

Sowing was carried out with manual mechanical seeder, in lines spaced 1m apart on October 30th, 2013. Seeding rate was 2.59kg ha^{-1} of viable pure seeds VPS. Seeds were treated with 0.07kg 100 kg^{-1} carboxin + 0.07kg 100kg^{-1} thiram and 0.0625L 100kg^{-1} fipronil, one day before sowing. At 30 days after emergence of plants, topdressing was carried out with N in the form of 75kg N ha^{-1} urea.

The experimental design was randomized blocks, with seven treatments, with four plots per treatment, and each plot consisted of 25m^2 (5x5m). Nitrogen doses, in the form of urea, constituted the treatments, and were applied at pre-flowering, corresponding to 180 days from sowing on May 6th, 2014 (doses of 0, 25, 50, 75, 100, 125 and 150kg ha^{-1}).

One meter lines (1m^2 each) from the useful plot were used for the collection of data related to: a) fully expanded inflorescences: evaluation was carried out weekly, and began when the first fully expanded inflorescences were observed. Evaluation finished at seed maturation and at the beginning of threshing, immediately prior to harvest; b) Yield of green and dry biomass of plants: the evaluation was carried out at flowering; and therefore, a sample of each useful plot was cut close to the ground; c) Chlorophyll (SPAD unit): measurement was carried out at full flowering. Chlorophyll content was measured using the portable chlorophyll meter Minolta SPAD-502 in 10 points per plot; d) Nutritional analysis of leaves: the third fully expanded leaf from the apex to the base of the plant was collected at full flowering, totaling approximately 200g green matter per sample and one sample per plot; e) At five fully-expanded inflorescences per plot immediately before threshing (seeds maturation), it was determined: number of racemes per inflorescence, maximum length of raceme, mean length of raceme, length of inflorescence axis (between the insertion point of the first to the last raceme in the inflorescence), and number of spikelets per raceme.

Harvest was carried out on June 17th, 2014, at maturation, when 15 to 20% seeds were threshed to touch, in the panicle (hand harvested seeds). The collected material was packed in paper bags and allowed to dry with approximate 10% water content. After drying, harvested seeds were benefited, and those which remained in the panicle were also taken out manually, and then samples were subjected to pre-cleaning and cleaning using metal sieves.

All the analyses related to the produced seeds were carried out with 8.5% mean water content, determined in accordance with the Rules for Seed Analysis, RAS (BRASIL, 2009). These analyses began in mid-September/2014, and the following variables were evaluated according to RAS (BRASIL, 2009): a) Yield and physical purity of pure seed; b) Tetrazolium test; c) Weight of thousand seeds; d) standard germination test; e) germination speed index, according to MAGUIRE (1962); f) first germination count (FGC%), seven day after the establishment of the test.

Immediately after seed harvesting , new soil samples were collected at 0-20cm depth for analysis.

Variables were subjected to analysis of variance, and treatments means were compared by the Duncan test at 5% probability. Statistical analyses were carried out using the SAS version 9.3 (SAS INSTITUTE INC, 2012) software.

RESULTS AND DISCUSSION

Flowering period of B4 genotype began in mid-May, and the first inflorescences fully expanded nine days after nitrogen fertilization of plants (Table 1). All plots reached full flowering (full anthesis with 20 inflorescences per square meter) 22 days after fertilization, occurring 13 days after the beginning of flowering, and seven months after sowing. There was variation in flowering in function of nitrogen fertilization, and the greatest value of fully expanded inflorescences per square meter occurred for the 50kg ha^{-1} dose, but differed only 25kg ha^{-1}, suggesting that the N content in the soil, for all treatments, was sufficient to meet the need of the forage plant in inflorescences emission, and the element was not considered a limiting factor for the test. Harvest was carried out 32 days after the beginning of flowering, when 15 to 20% seeds were threshed to touch. It is noteworthy that even in the absence of N application from external sources, flowering was satisfactory.

The organic matter content in the soil of the trial may have been enough to express the full potential of the accession as forage, and nitrogen fertilization carried out 30 days after emergence may also have contributed to this. Furthermore, for the genotype in question, the period between the beginning of floral differentiation, in which the fertilizer is applied, and harvest does not seem to be enough for the response to the nutrient. Moreover, in the region of Campo Grande-MS, B4 genotype has longer vegetative growth when compared to all other ***Brachiaria*** cultivars, and it also has late seed production. Flowering began in the first half of May, and harvest (panicle) was carried out in the second half of June (VERZIGNASSI, 2015, unpublished data). At this time of production, in dry season, weather conditions are not very favorable for nutrients responses, due to reduced rainfall index. HUMPHREYS & RIVEROS, (1986) refer to the production of seeds in response to fertilization as totally dependent on the temperature and moisture conditions at the time of nutrient application.

Regarding the vegetative phenological characters (Table 2) and green biomass yield, nitrogen fertilization provided no difference between treatments, which was contrary to dry biomass and dry matter values. Very high doses

Table 1 - Number of fully expanded inflorescences per square meter of ***Brachiaria brizantha*** B4 under different nitrogen doses, evaluated weekly. Campo Grande-MS, 2014.

N (kg ha^{-1})	---Evaluation dates---				
	15/05	21/05	28/05	04/06	11/06
0	0.00^{1,2}	6.75ab^{3}	49.00^{3}	85.50b^{3}	140.50ab^{3}
25	1.75	13.50ab	64.75	77.50b	110.25b
50	0.00	14.25ab	81.75	154.25a	199.75a
75	0.00	7.25ab	53.00	75.25b	137.50ab
100	0.00	3.00b	46.75	81.50b	145.00ab
125	0.00	13.75ab	76.50	101.75b	174.75ab
150	0.00	18.00a	69.75	91.75b	168.25ab
Mean	0.25	10.93	63.07	95.36	153.71
Treatment	1.00^{ns}	1.90*	0.91^{ns}	2.71*	1.34*
Block	2.31	1.70	2.10	0.53	0.83
CV(%)	68.57	39.60	23.97	15.81	16.12

^{1}Means of 4 replications. Mean followed by the same letter in the column do not differ by the Duncan test. *significant at 5% probability (0.01<P≤0.05), **significant at 1% probability (P=0.01), ^{ns}not significant (P>0.05). ^{2}Data transformed to $(x+0.5)^{1/2}$. ^{3}Data transformed to $(x)^{1/2}$. Data from the table are original.

Table 2 - Vegetative phenological characters: green biomass (GB), percentage of dry matter (DM), dry biomass (DB) and chlorophyll (CF) (SPAD unit) of ***Brachiaria brizantha*** B4; and reproductive phenology characters: number of racemes per inflorescence (NR), maximum length of raceme (LRMax), mean length of raceme (LRMean), length of inflorescence axis (distance between the insertion point of the first to the last raceme of the inflorescence) (LIA), and number of spikelets per raceme (SR) of ***Brachiaria brizantha*** B4 under different nitrogen doses. Campo Grande-MS, 2014.

N	GB	DB	DM	CF	NR	LTmax	LRmean	LIA	SR
(kg ha^{-1})	---------- (t ha^{-1})----------		(%)	--------(SPAD)---------		------------------------------ -(cm)-------------------------------			
0	42.80[1]	31.80ab	73.73ab	42.20	5.30	10.23	8.65	10.06	29.76abc
25	52.50	43.10a	82.69a	42.30	6.10	9.67	8.10	11.10	30.17abc
50	48.60	34.60ab	72.26ab	41.30	6.25	9.67	8.36	11.40	31.14ab
75	49.00	36.10ab	73.58ab	43.25	5.60	9.45	8.24	10.24	27.93c
100	34.60	25.30b	71.52ab	41.37	6.40	9.72	8.09	11.35	28.81bc
125	41.80	25.40b	63.66b	42.85	5.85	10.24	8.72	10.49	31.97a
150	46.50	31.00ab	69.33ab	43.37	5.90	9.68	8.44	11.02	29.33abc
Mean	45.11	32.47	72.40	43.38	5.91	9.81	8.37	10.81	29.87
Treat.	0.71^{ns}	1.63*	1.21*	0.34^{ns}	1.06^{ns}	1.62^{ns}	1.03^{ns}	0.80^{ns}	2.18*
Block	2.41	2.03	0.11	2.89	1.64	1.23	0.92	0.20	0.35
CV(%)	31.27	30.19	30.16	6.78	12.49	4.84	5.87	11.23	6.22

[1]Means of 4 replications. Mean number of plants per square meter: 3. Each replication consists of 5 inflorescences. Means followed by the same letter in the column do not differ by the Duncan test. *Significant at 5% probability (0.01<P≤0.05), ^{ns}not significant (P>0.05).

of nitrogen did not provide increase in dry matter yield. It is noteworthy that the values of dry matter were high for all the treatments, since plants were evaluated at full flowering. However, it should be mentioned that the plant has late flowering, and full flowering occurs in the dry season. For chlorophyll (SPAD unit), no differences were found between treatments.

Forage plants have variable tillering and, in the case of B4, there is the formation of a high percentage of dry matter, high C.N^{-1} ratio and low leaf:stem ratio, especially in the dry season. COSTA et. al. (2009) studied the effect of nitrogen fertilization in ***B. brizantha*** cultivars, which were divided into three applications, and reported positive results with the linear increase in N doses for dry matter production.

In relation to the reproductive phenological characters of ***B. brizantha*** B4 (Table 2), there were no differences between treatments, except for number of spikelets per raceme. These values, in general, seem to be more related to genetic inheritance than to the management of plants with fertilization. VALLE (2015, unpublished data), in trials of cultivar distinctiveness, reported mean values of inflorescence axis length of 11.18cm, basal raceme length of 10.75cm, racemes number of 5.47, spikelets number in the basal raceme of 34.07, and these values were very close to those reported in the present study. According to QUADROS et al. (2010), the raceme size is important information for forage species cultivars, and the greater the length of racemes, the largest is seed production ability of plants.

Results of leaf analysis for macro and micronutrients are described in table 3, and there were differences in response to nitrogen fertilization only for sulfur (S). Mean yield of pure seeds (Table 4) was higher with the increase of nitrogen doses to up to 50kg ha^{-1}, and then it was maintained at doses of 125 and kg ha^{-1} N. All treatments resulted in the same harvest point, so that no dose provoked early or late maturation.

For weight of a thousand seeds, the values obtained ranged from 7.09 to 7.77g and the lowest value was 150kg N ha^{-1}, differing only 50 and kg N ha^{-1}. Germination ranged from 7.59% to 15.82%, and the highest percentage corresponded to zero dose. For the highest dose (150kg N ha^{-1}), there was reduction of germination in relation to zero dose.

Nitrogen fertilization interfered with the viability of seeds, and the results ranged from 56-73% between treatments, leading to a reduction from the dose of 150kg ha^{1} N, suggesting reduction of seed quality at high N doses. The low percentage of germination is more related to dormancy than to the N doses used. This was verified by the high tetrazolium values and low germination percentage,

Table 3 - Results of leaf analysis for macronutrients and micronutrients of ***Brachiaria brizantha*** B4 under different nitrogen doses. Campo Grande-MS, 2014.

N	N	P	K	Ca	Mg	S	Fe	Mn	Zn	Cu	B
(kg ha^{-1})	g kg^{-1}						mg kg^{-1}				
0	14.14	1.03	17.32	3.72	2.86	1.22ab	198.64	55.30	14.11	4.68	11.13
25	15.96	1.14	9.35	3.83	2.75	1.22ab	215.60	56.98	13.88	4.93	11.43
50	14.45	1.13	11.86	3.95	3.02	1.44a	206.88	58.93	16.07	5.18	10.38
75	15.19	1.06	10.10	4.15	3.19	1.35ab	197.43	66.89	15.29	5.18	11.07
100	14.59	1.00	9.96	3.59	2.69	1.25ab	194.28	57.26	14.50	4.93	9.98
125	15.12	1.10	11.01	3.86	2.95	1.25ab	192.34	62.98	14.96	5.05	9.51
150	14.94	1.12	9.72	3.68	2.97	1.16b	180.71	56.70	14.44	4.68	9.98
Mean	14.91	1.08	11.33	3.83	2.92	1.27	197.98	59.29	14.75	4.95	10.50
Treatment	0.63^{ns}	0.76^{ns}	0.74^{ns}	0.92^{ns}	0.42^{ns}	1.69*	0.48^{ns}	0.99^{ns}	1.24^{ns}	1.07^{ns}	0.47^{ns}
Block	0.82	3.95	0.95	0.58	0.18	0.89	2.02	3.36	0.51	0.48	1.09
CV(%)	10.06	10.89	56.92	10.33	17.77	11.49	16.18	14.02	9.15	8.20	20.08

Means of 4 replications. Means followed by the same letter in the column do not differ by the Duncan test. *Significant at 5% probability ($0.01<P\leq0.05$).

which indicates the occurrence of dormancy. Also, seeds germination even after 10 months of storage, with values up to 19%, did not increase when compared to the results obtained in relation to September, 2014.

In the results of soil analysis obtained immediately after seeds harvest, potassium maintained the same value of 0.08cmol dm^{-3}, even after the addition of 70kg ha^{-1} K_2O before sowing. Phosphorus presented the same behavior, which shows that both elements were completely drained by the forage plant. Similarly, zinc (reduced to 2.42mg dm^{-3}) and boron (reduced to 0.22mg dm^{-3}) were the most demanded micronutrients by plants. The other elements remained on average in the same initial levels reported before sowing.

CONCLUSION

Nitrogen fertilization increases the parameters of yield components and the physiological performance of ***Brachiaria brizantha***

Table 4 - Yield of pure seeds (PS) in a population of 50,000 plants per hectare; weight of a thousand seeds (WTS), physical purity (PP), germination (G), first germination count (FGC), germination peed index (GSI), viability by the tetrazolium test (TZ), and germination of ***Brachiaria brizantha*** B4 seeds at 10 months after harvest (G10), under different nitrogen doses. Campo Grande-MS, 2014.

N (kg ha^{-1})	PS (kg ha^{-1})	WTS (g)	PP (%)	G (%)	FGC (%)	GSI	TZ (%)	G10 (%)
0	86.10bc[1,2]	7.54ab	20.89	15.82[3]a	2.01[4]	1.50[5]a	62.00ab	14.25ab
25	87.60bc	7.60ab	22.62	11.63ab	0.21	0.94ab	73.00a	19.00a
50	144.80a	7.67a	26.47	8.65ab	0.43	0.78ab	61.37ab	11.50ab
75	79.60c	7.77a	20.81	14.51ab	0.06	1.06ab	66.87ab	17.00ab
100	83.80c	7.32ab	18.405	8.36ab	0.00	0.70b	67.75a	12.00ab
125	116.70ab	7.36ab	22.825	9.52ab	1.57	0.91ab	64.62ab	7.75b
150	139.80ab	7.09b	26.38	7.59b	0.88	0.70b	56.00b	14.00ab
Mean	105.49	7.48	22.63	10.87	0.74	0.94	64.52	13.64
Treatment	3.32*	1.89*	-	1.58*	1.11^{ns}	1.37*	2.39*	1.84*
Block	6.67	1.76	-	4.95	2.74	6.97	0.92	3.07
CV(%)	14.12	4.51	-	24.59	49.67	15.60	10.89	38.36

[1]Means of 4 replications. Means followed by the same letter in the column do not differ by the Duncan test. [2]Data transformed to $(x)^{1/2}$. [3]Data transformed to arcsin $(x+0.5/100)^{1/2}$. [4]Data transformed to $(x+0.5/100)^{1/2}$. [5]Data transformed to $(x+0.5)^{1/2}$. Data in Table are original. *Significant at 5% probability ($0.01<P\leq0.05$), ^{ns}not significant ($P>0.05$). Data in table are original.

B4 seeds. The maximum yield of pure seed was 144.8kg ha^{-1} for the dose of 50kg ha^{-1} N. Based on seed yield components, the maximum biological potential in yield of pure seed was 456.27kg ha^{-1}. Seeds dormancy of the genotype was higher, and it took no longer than 10 months after storage.

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